Suboptimal light conditions negatively affect the heterotrophy of Planktothrix rubescens but are beneficial for accompanying Limnohabitans spp

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Abstract: We examined the effect of light on the heterotrophic activity of the filamentous cyanobacterium Planktothrix rubescens and on its relationship with the accompanying bacteria. In situ leucine uptake by bacteria and cyanobacteria was determined in a subalpine mesotrophic lake, and natural assemblages from the zone of maximal P. rubescens abundances were incubated for 2 days at contrasting light regimes (ambient, 100× increased, dark). Planktothrix rubescens from the photic zone of the lake incorporated substantially more leucine, but some heterotrophic activity was maintained in filaments from the hypolimnion. Exposure of cyanobacteria to increased irradiance or darkness resulted in significantly lower leucine incorporation than at ambient light conditions. Highest abundances and leucine uptake of Betaproteobacteria from the genus Limnohabitans were found in the accompanying microflora at suboptimal irradiance levels for P. rubescens or in dark incubations. Therefore, two Limnohabitans strains (representing different species) were co-cultured with axenic P. rubescens at different light conditions. The abundances and leucine incorporation rates of both strains most strongly increased at elevated irradiance levels, in parallel to a decrease of photosynthetic pigment fluorescence and the fragmentation of cyanobacterial filaments. Our results suggest that Limnohabitans spp. in lakes might profit from the presence of physiologically stressed P. rubescens.

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Suboptimal light conditions negatively affect the heterotrophy of *Planktothrix rubescens* but are beneficial for accompanying *Limnohabitans* spp.

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Summary

We examined the effect of light on the heterotrophic activity of the filamentous cyanobacterium *Planktothrix rubescens* and on its relationship with the accompanying bacteria. *In situ* leucine uptake by bacteria and cyanobacteria was determined in a subalpine mesotrophic lake, and natural assemblages from the zone of maximal *P. rubescens* abundances were incubated for 2 days at contrasting light regimes (ambient, 100× increased, dark). *P. rubescens* from the photic zone of the lake incorporated substantially more leucine, but some heterotrophic activity was maintained in filaments from the hypolimnion. Exposure of cyanobacteria to increased irradiance or darkness resulted in significantly lower leucine incorporation than at ambient light conditions. Highest abundances and leucine uptake of *Betaproteobacteria* from the genus *Limnohabitans* were found in the accompanying microflora at suboptimal irradiance levels for *P. rubescens* or in dark incubations. Therefore, two *Limnohabitans* strains (representing different species) were co-cultured with axenic *P. rubescens* at different light conditions. The abundances and leucine incorporation rates of both strains most strongly increased at elevated irradiance levels, in parallel to a decrease of photosynthetic pigment fluorescence and the fragmentation of cyanobacterial filaments. Our results suggest that *Limnohabitans* spp. in lakes might profit from the presence of physiologically stressed *P. rubescens.*

Introduction

Solar radiation, particularly its photosynthetically active and UV components, affects growth and production of photosynthetic energy-harvesting enzymes (e.g. Vassiliev *et al*., 1994) as well as DNA synthesis and photosynthetic pigment contents (e.g. Buma *et al*., 1995; Gerber and Häder, 1995). Phototrophic aquatic organisms typically face rapidly changing conditions both, in terms of light quantity and quality and with respect to the depth of light penetration through the water column. Many algae and cyanobacteria have developed strategies to reduce the negative effects of excessive radiation, including the avoidance of brightly irradiated habitats, the synthesis of UV-screening pigments, or the removal of photochemically produced radicals (Vincent and Roy, 1993). Other adaptations of phytoplankton organisms to variable light intensity involve changes in the pigment composition and content (e.g. Takahashi *et al*., 1989; Falkowski and La Roche, 1991) or in light absorption efficiency (Duysens, 1956).

Besides the purely photoautotrophic aquatic bacteria, there are also photoheterotrophic taxa capable of both, harvesting light energy and utilizing organic compounds (e.g. Eiler, 2006). Photoheterotrophy occurs in a broad range of microbes including aerobic anoxygenic phototrophic bacteria (Kolber *et al*., 2000), proteorhodopsin-containing bacteria (Beja *et al*., 2000) and cyanobacteria (e.g. Chisholm *et al*., 1988). Although the latter are primarily photoautotrophic, some species can grow even in the dark on various organic compounds such as sugars, organic acids or amino acids (e.g. Fay, 1965; Hoare *et al*., 1967; Smith *et al*., 1967). Such assimilation of organic substrates can substantially support the growth requirements of cyanobacteria at very low or fluctuating irradiance levels below the photosynthetic compensation point (Pearce and Carr, 1966; Fogg *et al*., 1973).

Planktothrix rubescens (Anagnostidis and Komárek, 1988) is a filamentous cyanobacterium that dominates the photoautotrophic community of the large mesotrophic subalpine Lake Zürich, Switzerland (Micheletti *et al*., 1998; Van den Wyngaert *et al*., 2011). *P. rubescens* is buoyant via gas vesicles and thus reveals a very distinct vertical distribution pattern during periods of stable stratification during summer and autumn: it forms very dense and persistent populations in the metalimnion of the lake, whereas only few filaments are present in the epilimnion and, to an even lesser extent, in the hypolimnion. While *P. rubescens* is adapted to the low quantities of light typically found in the metalimnion, the irradiance conditions in this zone may nevertheless not be sufficient to sustain its growth. However, it is able to incorporate substrates such as acetate and amino acids to partially cover the carbon and nitrogen demands, and the assimilation of the substrates is enhanced at very low light levels (Zotina *et al*., 2003; Walsby and Jüttner, 2006). This photoheterotrophy allows *P. rubescens* to outcompete other phytoplankton organisms (Walsby *et al*., 2004).

Cyanobacterial blooms affect the development of heterotrophic bacteria (Van Hannen *et al*., 1999; Van den Wyngaert *et al*., 2011). Some bacteria may benefit from the presence of cyanobacteria, e.g. by colonizing their mucilage and utilizing their extracellular release (Eiler *et al*., 2006), whereas others are negatively affected by cyanobacterial secondary metabolites with toxic or antibacterial properties (e.g. Østensvik *et al*., 1998). Previous studies have reported that members of *Alphaproteobacteria* (*Sphingomonas* sp.) profited from a bloom of *Microcystis* spp. (Park *et al*., 2001; Maruyama *et al*., 2003) whereas *Betaproteobacteria* related to *Limnohabitans* sp. were negatively affected (Horňák *et al*., 2008). Knowledge about the interaction of *P. rubescens* with heterotrophic bacterioplankton is scarce. While these cyanobacteria are known to produce microcystins that are highly toxic to zooplankton grazers (Blom *et al*., 2001) their

82 release of extracellular compounds is very limited (Feuillade *et al*., 1990). Recently, a detailed
83 investigation on the seasonal distribution of bacteria and *P. rubescens* in Lake Zürich showed
84 that some bacterial genotypes related to *Fluviicola* sp. (*Cytophaga-Flavobacteria*) formed the
85 largest populations in the layer of maximal *P. rubescens* abundances (Salcher *et al.,* 2011).
86 The goal of our study was to examine the effects of light on (i) the photoheterotrophy of
87 *P. rubescens* and (ii) the relationship between this cyanobacterium and heterotrophic bacteria.
88 We hypothesized that some bacterioplankton groups might be favored if *P. rubescens* was
89 exposed to suboptimal levels of irradiance or to darkness, as encountered in the epi- and
90 hypolimnion of the lake, respectively.
91
92 **Results**
93 In situ *depth profiles*
94 Lake Zürich was vertically stratified at the sampling time point (Fig. 1). The warm
95 epilimnetic layer with a surface temperature of 19.2 °C reached down to a depth of 12 m. The
96 thermocline was located between 12 to 15 m, followed by a continuous decrease in temperature
97 to 7.3 °C at a depth of 30 m. Oxygen concentrations were virtually uniform in the epilimnion, but
98 there was a strong metalimnetic oxygen depletion between 13 to 15 m followed by a slight
99 increase in deeper water layers. Photon irradiance gradually decreased down to a depth of 10 m
100 (Fig. 1) and it was then disproportionally reduced, likely due to the high abundances of *P.*
101 *rubescencs* in the metalimnion (Fig. 1). The average irradiance experienced by microbes within
102 the euphotic zone of the water column accounted for 230 µmol m^{-2} s^{-1} which was approximately
103 two orders of magnitude higher than in the metalimnion.

Total bacterial numbers varied from 1.6 to 4.5 × 10^6 cells ml^{-1} within the upper 30 m (Fig. 1). Maximal bacterial abundances were detected between 5 and 7.5 m, and gradually decreased below this depth. While only very low cell abundances of *P. rubescens* (< 25 filaments ml^{-1}) were encountered in the epilimnion, the cyanobacterial population was stratified in high concentrations (> 300 filaments ml^{-1}) at the depth of 12.5 m (Fig. 1). High *P. rubescens* abundances (~150 filaments ml^{-1}) were still encountered at the transition zone between the meta- and the hypolimnion, followed by a marked decline in deeper water strata. Picocyanobacteria were mainly present in the epilimnion while they were not detected below a depth of 15 m.

Quantitative leucine incorporation by P. rubescens

Unfortunately, a simple size-fractionation of the raw water sample by filtration (5 µm pore size) was not sufficient to accurately separate the leucine incorporation by *P. rubescens* from that of the accompanying microbial community (Fig. S1) due to the presence of bacterial filaments and colonial cyanobacteria that also incorporated leucine in the > 5 µm fraction (Fig. S2). Therefore, a specific procedure was developed that is based on the relationship between the amount of incorporated leucine by individual *P. rubescens* filaments and the filament area that is covered by silver grains after microautoradiography (MAR) (Fig. S2). In lake samples from 2.5 m and 12.5 m depth, the MAR+ filament area (i.e., the fraction of total filament area covered by silver grains) of the majority of filaments varied between 14 to 19 %, whereas a substantially lower MAR+ filament area was observed in samples from 30 m (Fig. 2). Moreover, the population of *P. rubescens* in the hypolimnetic sample was physiologically more heterogeneous than in the epi- and metalimnion, as indicated by a large scatter of filament activity. In addition, the MAR+ filament area in almost half of the filaments from a depth of 30 m was as low as in the

126 negative controls, suggesting that there was no active leucine incorporation in a large part of the
127 hypolimnetic *P. rubescens* population.

128 Leucine incorporation by *P. rubescens* was also observed in experimental incubations
129 where the natural community from a depth of 12.5 m was exposed to different irradiance regimes
130 (Fig. 3). At the beginning of the experimental manipulation, MAR+ filament area accounted for
131 approximately one fifth of total filament area, comparable to the field situation in the epi- and
132 metalimnion (Fig. 2). However, the amount of leucine incorporated into *P. rubescens* filaments
133 significantly decreased after 45 h of incubation at both, increased irradiance levels and in the
134 dark, while no such decrease was observed in samples incubated at ambient irradiance (Fig. 3). In
135 all negative controls, MAR+ filament area varied around 5 %.

136 In situ *bacterial community composition and leucine incorporation*

137 CARD-FISH analyses of the percentages of major phylogenetic groups of bacteria in
138 samples from three selected depths revealed a high similarity in community composition (Table
139 1). In all cases, the proportions of *Actinobacteria* varied around 20 % of total DAPI-stained cells,
140 *Betaproteobacteria* accounted for about 10 %, and slightly lower proportions of *Cytophaga-*
141 *Flavobacteria* were found (Table 1). Two subgroups within *Betaproteobacteria* were analyzed:
142 members of the R-BT065 lineage (*Limnohabitans* spp.) (Table 1) and of the *Polynucleobacter*
143 cluster (data not shown) accounted for about 2.5 % and 2 % of DAPI-stained cells, respectively.

144 All studied bacterial groups in the lake samples incorporated leucine (Table 1). Both,
145 *Betaproteobacteria* and members of the R-BT065 lineage, displayed decreasing proportions of
146 cells with visible leucine incorporation (Leu+ cells) along the depth profile, ranging from 72 to
147 56 % and 93 to 71 %, respectively. In contrast, percentages of Leu+ *Actinobacteria* gradually
148 increased with increasing depth (53 to 63 %). Only less than 10 % of *Cytophaga-Flavobacteria*

were found to incorporate leucine (Table 1). In three studied depths, both relative abundances and proportions of bacteria incorporating leucine covered by a set of EUB338I-III probes were statistically indistinguishable and ranged from 63 to 60 % and from 53 to 47 %, respectively.

Bacterial community composition and leucine incorporation in incubations

Although bacterial numbers in all treatments slightly decreased ($3.3 – 3.7 \times 10^6$ cells ml^{-1}) compared to the initial time point (3.8×10^6 cells ml^{-1}), there were no statistically significant changes in total bacterial abundances. By contrast**,** significant differences in bacterial community composition and in the proportions of cells with visible leucine incorporation were observed. *Betaproteobacteria* were significantly more abundant only in treatments with increased irradiance (Fig. 4). The relative abundances of *Actinobacteria* and of members of the *Cytophaga-Flavobacteria* revealed only few significant differences after 45 h of incubations (Table 2). *Actinobacteria* showed increased proportions of Leu+ cells at increased irradiance while *Cytophaga-Flavobacteria* were excluded from the analysis due to very low proportions of MAR+ cells and no pronounced changes in its relative abundances compared to the initial sample (Table 2). In all treatments, the relative abundances of bacteria targeted by the probe R-BT065 (*Limnohabitans* spp.) significantly increased over time, whereas the percentage of Leu+ cells in this group was significantly elevated only in the dark incubations (Fig. 4).

Impact of different irradiance regimes on P. rubescens *and* Limnohabitans *strains*

The same irradiance regimes as in the incubation of natural assemblages were used in experiments with axenic strains of *P. rubescens, L. parvus* and *L. planktonicus*. In pure cultures of *P. rubescens* and in co-cultures with *Limnohabitans* bacteria*,* significant differences in phycoerythrine and chlorophyll *a* fluorescence were observed after 169 h at different irradiance

conditions (Fig. 5, Table S1). Moreover, the mean filament length of *P. rubescens* at increased irradiance was in most cases significantly smaller, resulting in higher filament abundances and subsequently in changes in total cyanobacterial biomass in the respective pure and co-culture treatments (Table 3). Leucine incorporation rates of *P. rubescens* increased in all treatments, and this was most pronounced at ambient irradiance conditions (Fig. 5). However, nearly all differences in leucine incorporation rates at different light conditions at the end of the incubation period were statistically indistinguishable (Table S1).

In pure culture, *L. planktonicus* reached higher abundances and leucine incorporation rates after 169 h at ambient irradiance levels and in the dark treatments than at increased irradiance, as compared to initial values (Fig. 6). By contrast, the final abundances of *L. parvus* only slightly increased in these treatments, and leucine incorporation rates even decreased (Fig. 6). Both strains, particularly *L. parvus*, reached distinctly higher cell abundances after 169 h in co-cultures with *P. rubescens* as compared to pure cultures (Fig. 6). Moreover, in co-cultures with *P. rubescens*, significant differences in abundance and leucine incorporation rates of *Limnohabitans* strains at different irradiance levels were detected (Table S2). The highest abundances of both bacterial strains in co-cultures were found at increased irradiance (Fig. 6). This was also the case for bacterial leucine incorporation rates, whereas the uptake of this amino acid at ambient irradiance levels and in the dark was comparable to the pure cultures.

Discussion

In situ *heterotrophic activity of* P. rubescens

In order to accurately study the heterotrophy of *P. rubescens* in situ, it was essential to go beyond the mere determination of bulk amino acid incorporation after size fractionation (Fig. S1),

since this procedure could not distinguish between the uptake caused by *P. rubescens* and by other microorganisms in the same size fraction (Fig. S2). A reproducible and quantitative analysis of cyanobacterial tracer incorporation at close to *in situ* conditions could be realized by combining microautoradiography with image analysis, as has been previously used to quantify the substrate assimilation in planktonic bacteria (e.g. Cottrell and Kirchman, 2003) or in filamentous bacteria in an activated sludge system (Nielsen *et al.,* 2003). As compared to the latter study we did not use an internal standard consisting of cells with known radioactivity since the absolute uptake of substrate was not determined but instead relative differences in the substrate uptake activity were compared. We, nevertheless, applied controlled MAR conditions to minimize variability in the formation of silver grains caused by the thickness of the emulsion, exposure time or by variability between individual MAR preparations (Nielsen *et al.,* 2003). Despite the above mentioned factors influencing the formation of grains we could accurately determine and quantify the leucine assimilation of *P. rubescens* by our specific approach (Figs. 2, 3).

Although a clear maximum of *P. rubescens* was detected in the metalimnion (Fig. 1), leucine incorporation of the filaments was comparable in the epi- and the metalimnion (Fig. 2). This suggests that the heterotrophic activity of *P. rubescens* in the epilimnion was not significantly different from the one of the metalimnetic population, even though cyanobacteria were not exposed to optimal light conditions in the epilimnion. Our findings are thus in contrast with earlier results that showed a dependency of the heterotrophic uptake of organic substrates by *P. rubescens* on light conditions in pure cultures and in epilimnetic populations after deepening of the surface mixed layer (Zotina *et al*., 2003). However, the heterotrophic metabolism of *P. rubescens* might not respond immediately to increased light quantities. It is conceivable that the

217 filaments investigated in our study were not permanently subjected to the enhanced irradiance of
218 the upper water layers. Instead, they might have originated from the metalimnetic population and
219 temporarily entrained in the epilimnion by mixis within the thermally homogenous upper 13 m of
220 the water column. Moreover, our *in situ* data on leucine uptake activities (Fig. 2) suggest that
221 cyanobacterial filaments were light-saturated already in the metalimnion. A prolonged exposure
222 of *P. rubescens* to increased light conditions indeed resulted in significantly decreased amino
223 acid incorporation also in our experimental setup (Fig. 3).

224 *P. rubescens* typically grows at low levels of photosynthetically active irradiances (Foy *et*
225 *al.*, 1976; Davis and Walsby, 2002), and it can even survive in complete darkness for a short time
226 period (Gibson, 1975). Our MAR analyses revealed that the population of *P. rubescens* sampled
227 from a depth of 30 m (i.e., the aphotic zone, Fig. 1) was very heterogeneous in terms of
228 heterotrophic activity (Fig 2): While some filaments in this layer were clearly active, a substantial
229 proportion (approximately 50%, as indicated by the median value) was indistinguishable from the
230 prefixed controls. Thus, the range of activity in 30 m was substantially higher than in the other
231 two layers. This suggests that a subpopulation of *P. rubescens* filaments was able to maintain
232 amino acid incorporation after sedimentation into the aphotic zone. Moreover, we were able to
233 detect individual filaments with visible leucine incorporation even in the depth of 60 m (data not
234 shown), which is still above the critical depth for the survival of *P. rubescens* (Walsby and
235 Schanz, 2002). Our results are consistent with those of Holland and Walsby (2008) who also
236 showed viability of different *P. rubescens* strains exposed to conditions prevailing in the aphotic
237 zone. The heterotrophic incorporation of organic substrates in the dark hypolimnion may prolong
238 the survival of these cyanobacteria under unfavorable conditions, as induced by autumnal mixing

239 of the water column (Zotina *et al*., 2003; Walsby and Jüttner, 2006) and allow for their eventual
240 ascend into the metalimnion after the onset of thermal stratification (Holland and Walsby, 2008).

241 *Response of* P. rubescens *to different irradiance regimes*

242 The prior determination of basic physicochemical and biological parameters *in situ* (Fig.
243 1, Table 1) provided a framework for the setup and interpretation of laboratory experiments with
244 a natural population of *P. rubescens* that was pre-adapted to the irradiance levels of the
245 metalimnion. Such conditions are regarded optimal for both, the purely autotrophic growth of *P.*
246 *rubescens* (Micheletti *et al*., 1998; Bright and Walsby, 2000) as well as its photoheterotrophic
247 activity (Zotina *et al*., 2003), e.g., the incorporation of amino acids (e.g. Walsby and Jüttner,
248 2006). The simulation of suboptimal irradiance regimes, as encountered by *P. rubescens* in the
249 epi- and hypolimnion allowed an assessment of the effects of light levels on cyanobacterial
250 photoheterotrophy (Fig. 3) and to reveal treatment-dependent shifts in the composition and
251 metabolic activity of the accompanying bacterial assemblage (Fig. 4, Table 2). However, it
252 should be noted that the experimental setup differed from the field situation, e.g. there was no
253 natural light-dark cycle; thus, irradiance-related effects were potentially magnified.

254 Besides temperature, both, the quality and quantity of light have been recognized as major
255 factors influencing the development of *P. rubescens* (e.g. Walsby and Schanz, 2002; Oberhaus *et*
256 *al*., 2007). Our experimental incubations at different irradiance intensities clearly affected the
257 heterotrophic metabolic activity of *P. rubescens*: Permanent exposure to either enhanced
258 irradiance or to darkness for 45 h induced a significant reduction in leucine incorporation by *P.*
259 *rubescens* compared to the initial levels, whereas no difference was found in incubations at the
260 ambient light conditions of the metalimnion (Fig. 3). Interestingly, the levels of leucine
261 incorporation by *P. rubescens* after 45 h of incubations in the dark closely resembled the activity

of the *in situ* population at a depth of 30 m (Fig. 2), suggesting that this part of our experimental setup adequately mimicked the field situation. However, as already outlined above, this might not hold true for the incubations at permanently enhanced levels of irradiation: While the experiments indicated a decrease in the heterotrophic activity of *P. rubescens* under such conditions (Fig. 3), this was not the case for filaments thriving in the epilimnetic layer of the lake (Fig. 2). Nevertheless, our experimental data suggested that the heterotrophic metabolic activity of optimally adapted *P. rubescens* was sensitive to prolonged exposure to suboptimal levels of irradiance, thus providing experimental confirmation of earlier field observations (Zotina *et al.*, 2003).

Effects of experimental manipulations on bacterioplankton

Although we observed only minor vertical changes in the proportions of major bacterial groups *in situ* (Table 1), our experimental manipulations clearly induced treatment-specific shifts in bacterial community composition. The relative abundance of *Betaproteobacteria* approximately doubled at increased irradiance (Fig. 4), as compared to their initial proportions. By contrast, the fraction of *Actinobacteria* was significantly higher after 45 h of incubation in the dark (Table 2), even though there are indications that these bacteria might directly profit from light via actinorhodopsins (Sharma *et al.*, 2008). Since the total bacterial counts barely changed during the incubation period - likely due to the simultaneous action of top-down factors (Pernthaler, 2005) - these changes in relative abundances can be directly translated into rises of cell numbers.

Bacteria from the R-BT065 lineage (*Limnohabitans* sp.) significantly increased in all experimental treatments (Fig. 4). These bacteria are known to rapidly respond to altering growth conditions in general (Šimek *et al.*, 2005; 2006). Thus, their success likely also reflected an

285 unspecific response to the manipulation of transferring water samples into the laboratory.
286 However, substantially higher fractions of leucine-incorporating R-BT065 cells were only
287 observed in the dark incubations (Fig. 4), indicating that these bacteria additionally benefitted
288 from the presence of high concentrations of physiologically stressed *P. rubescens* filaments. By
289 contrast, there were significantly less MAR-positive R-BT065 bacteria in the aphotic zone of the
290 lake than in epi- or metalimnetic waters (Table 1), which might be due to the considerably lower
291 ambient temperatures and cyanobacterial concentrations in that layer (Fig. 1) than in our dark
292 incubations.

293 Our findings thus document that distinct light regimes influenced the relative abundances
294 or the proportions of metabolically active cells of some heterotrophic lake bacteria. The most
295 significant changes were found in treatments with suboptimal light conditions for *P. rubescens,*
296 as reflected in their reduced heterotrophic metabolic activity (Fig. 3). This suggests that some
297 bacteria profited from the physiological condition of cyanobacteria that were exposed to 100-
298 times increased light intensity or to complete darkness. *P. rubescens* is well adapted to
299 microphotic conditions and its pigment composition (e.g. Bright and Walsby, 2000) allows for an
300 efficient acquisition of low quantities of light. On the other hand, it is sensitive to high quantities
301 of light, particularly to the green parts of the spectrum, and may be easily photoinhibited
302 (Oberhaus *et al.*, 2007). We, moreover, observed that treatments with elevated irradiance
303 intensities induced a pronounced aggregation of cyanobacterial filaments (data not shown), which
304 again indicates that these conditions were highly unfavorable for *P. rubescens* growth.

305 *Link between* P. rubescens *and* Limnohabitans *strains*

306 Since members of the R-BT065 lineage (that encompasses various *Limnohabitans* spp.)
307 showed a treatment-specific response in leucine incorporation (Fig. 4) and cultured

representatives from this clade are available (Kasalický *et al.*, 2010), we conducted a follow-up experiment using an axenic *P. rubescens* strain and two bacterial strains, *Limnohabitans parvus* and *L. planktonicus.*

Both bacteria obviously profited from the presence of *P. rubescens,* as reflected in significantly higher total cell numbers in the co-cultures than in the pure cultures (Fig. 6). However, the two strains revealed striking differences in their responses to the manipulation of light conditions: While total cell abundances of *L. parvus* in pure culture only increased slightly irrespective of the treatment, *L. planktonicus* reached more than one order of magnitude higher abundances at low light levels and in the darkness (Fig. 6, Table S2). These results might indicate that the growth of the latter strain was negatively affected by photoinhibition, as has been observed for bacterioplankton in the surface layers of mountain lakes (Sommaruga *et al*., 1997). At the same time, *L. planktonicus* much more efficiently utilized the trace levels of organic carbon available in the cultivation medium, which might hint at a niche separation between the two species (Šimek *et al*., 2010).

Irrespective of such differences, the largest positive and significant changes in abundances and in metabolic activity of both bacterial strains were found in co-cultures incubated under increased irradiance (Fig. 6). In this treatment *P. rubescens* moreover featured significantly decreased fluorescence of both, phycoerythrine and chlorophyll *a* pigments irrespective of the presence of bacteria (Fig. 5, Table S1), implying severe stress of cyanobacteria. This was, moreover, accompanied by a pronounced fragmentation of *P. rubescens* filaments, as reflected in a significantly shorter mean filament length than in the other treatments (Table 3). In contrast to other phytoplankton groups with high carbon exudation rates, e.g. cryptophytes (Giroldo *et al*., 2005), *P. rubescens* usually does not excrete a large amount of extracellular products (Feuillade

331 *et al*., 1990). Thus, the additional bacterial growth in co-cultures at elevated light irradiation was
332 likely due to substrates released into the medium upon the disintegration of filaments. This
333 conclusion is also supported by the substantially elevated leucine incorporation rates of both
334 bacterial strains (Fig. 6) in this treatment only.

335 In summary, we could show a beneficial effect of *P. rubescens* grown under suboptimal
336 light conditions on *Limnohabitans* spp. both in mixed assemblages and in pure co-culture.
337 Nevertheless, there were also obvious differences in the response of these bacteria between the
338 two experimental approaches, e.g., with respect to the most favorable treatment type (elevated
339 light or darkness), as well as between the two tested bacterial strains. Therefore, the relationship
340 between *Limnohabitans* spp. and light-stressed *P. rubescens* in natural assemblages might be
341 affected by co-occurring bacterial competitors, but also by the physiological differences between
342 individual *Limnohabitans* species.

343

344 **Experimental procedures**

345 *Study site, physicochemical parameters and sampling*

346 Lake Zürich (Switzerland; 406 m a.s.l.; area, 66.8 km^2; volume, 3.34 km^3; length, 29.1
347 km; max. depth, 136 m; mean depth, 51 m; mean retention time, 1.19 year) is a prealpine
348 mesotrophic lake characterized by the massive periodic development of the filamentous
349 cyanobacterium *Planktothrix rubescens*. In the second half of the year, *P. rubescens* stratifies in
350 the metalimnion, thereby forming a subsurface chlorophyll maximum layer at the border of the
351 photic zone. On 17 Sept 2007, a depth profile of 30 m close to the maximal depth area in the
352 central part of the lake was sampled at 2.5 m intervals. Two L of samples from every depth were
353 stored in light-tight bottles. Depth profiles of temperature and oxygen concentration were

measured *in situ* with a multipurpose probe (WTW, Germany). *In situ* photosythetically active radiation was measured using a spherical quantum sensor (LI-COR, USA). Total cell numbers of bacteria, *P. rubescens* and picocyanobacteria were analysed in all samples from the depth profile, whereas assays for bacterial community composition and incorporation of organic substrates were performed with samples from 2.5, 12.5 and 30 m depth. In addition, a total volume of ~40 L of water was collected from a depth of 12.5 m, where the *P. rubescens* chlorophyll maximum was located. This water was prefiltered through a plankton net to remove larger zooplankton (240 µm mesh size) and processed for the laboratory incubations within 2 h after sampling.

Experimental incubations of total microbial assemblages

Water sample from the depth of 12.5 m (*P. rubescens* maximum) was distributed into sterile glass bottles (each containing 1.8 l of water). All treatments were continuously incubated at *in situ* temperature for 45 h under (1) ambient (~2 µmol m^{-2} s^{-1}) and (2) 100x increased (~200 µmol m^{-2} s^{-1}) irradiance and (3) in the dark. Irradiance intensities were set to roughly correspond to natural irradiance intensities measured at the depths of 12.5, 2.5, and 30 m, respectively. All treatments were incubated in triplicates. Subsamples were taken before and after 45 h of incubations.

Experimental incubations in pure cultures and defined co-cultures

Planktothrix rubescens strain BC 9307 (Bristol Collection) is a filamentous cyanobacterium isolated from Lake Zürich in 1993. *Limnohabitans parvus* and *Limnohabitans planktonicus* (Kasalický *et al*., 2010) are fast-growing rod-shaped bacteria affiliated with the new genus *Limnohabitans* (Hahn *et al*., 2010) within the R-BT065 subcluster of *Betaproteobacteria* (Šimek *et al*., 2001). Bacterial strains were isolated from the surface layer of the freshwater

meso-eutrophic Římov reservoir (Czech Republic). Partial sequences of the 16S rRNA gene of *L. parvus* and *L. planktonicus* have been deposited in the GenBank (accession numbers FM165536 and FM165535, respectively).

Prior to the experiment, *P. rubescens*, *L. parvus*, and *L. planktonicus* were separately pre-cultivated at 17°C in inorganic artificial lake water (ALW) (Zotina *et al*., 2003) supplemented with equal amounts of nutrient broth, peptone, and yeast extract (total concentration of organic supplements, 9 mg l^{-1}), and with thiaminiumdichloride, biotin, and vitamin B12 (final concentration of each vitamin, 7.5 nM). Bacterial strains grew for 2 days in the dark while *P. rubescens* grew for 14 days at ~2 µmol m^{-2} s^{-1} (ambient irradiance) at a light/dark cycle of 14:10 h. Before starting the experiment, the abundances of bacteria and of *P. rubescens* filaments were determined by epifluorescence microscopy. Bacterial strains and *P. rubescens* were subsequently inoculated into 50 ml of fresh ALW in 150 ml Erlenmeyer flasks to initial cell abundances of approximately 1×10^6 cell ml^{-1} and 1×10^4 filaments ml^{-1}, respectively. Pure cultures of *P. rubescens*, *L. parvus*, and *L. planktonicus* as well as co-cultures of *P. rubescens* with either *L. parvus* or *L. planktonicus* were grown in triplicates for 169 h at 17°C either under continuous irradiance of ~200 µmol m^{-2} s^{-1} (increased), of ~2 µmol m^{-2} s^{-1} (ambient), and in the dark.

Abundances of bacteria, P. rubescens, *and of* Limnohabitans *strains*

Field samples for the determination of bacterial and *P. rubescens* abundance were fixed with freshly prepared buffered paraformaldehyde (PFA) (final concentration, 1%). Microbes were stained with 4´,6-diamidino-2-phenylindole (DAPI) (final concentration, 1 µg l^{-1}). Bacteria were concentrated on 0.2 µm polycarbonate filters (type GTTP, diameter 25 mm, Millipore) and filaments of *P. rubescens* on 5 µm polycarbonate filters (type GTTP, diameter 25 mm,

398 Millipore), respectively. Bacteria, *P. rubescens*, and coccoid cyanobacteria (if present), were
399 enumerated using epifluorescence microscopy (Axioskop, Zeiss, Germany).
400 One ml of sample from the pure cultures and defined co-cultures was fixed by PFA (final
401 concentration, 2 %) immediately after sampling. Samples were stained with the fluorochrome
402 DAPI (final concentration 1 µg ml^{-1}) and *Limnohabitans* strains were counted using the Influx V-
403 GS flow cytometer (Becton Dickinson, USA) equipped with a UV-laser (Lightwave Electronics,
404 CY-PS, 60 mW, 355 nm). Latex microspheres (Polyscience, FlowCheck® High Intensity Green
405 Alignment, diameter 1.0 µm) were used as an internal standard. Bacteria were identified in a plot
406 of 90° angle light scatter versus DAPI fluorescence (460 nm) using the flow cytometry analysis
407 software FlowJo (Tree Star, USA).

408 *Bacterial identification and microautoradiographic analysis*
409 Bacterial community composition and the leucine incorporation by probe-defined
410 bacterial populations were tracked by catalyzed reporter deposition-fluorescence *in situ*
411 hybridization (CARD-FISH) (Sekar *et al*., 2003) combined with microautoradiograhy (MAR)
412 (Teira *et al*., 2004). MAR was also applied to analyze leucine uptake by *P. rubescens* filaments.
413 Triplicate samples and paraformaldehyde-fixed blanks of 5 ml were incubated with L-[^{3}H]-
414 leucine (Leu, final concentration 10 nmol l^{-1}, specific activity 2.55 TBq $mmol^{-1}$, Amersham) for 2
415 h at *in situ* temperature and roughly ambient irradiance conditions. Incubations were terminated
416 by adding PFA (final concentration, 1 %). Bacteria and filaments of *P. rubescens* were
417 subsequently concentrated on 0.2 and 5 µm polycarbonate filters (type GTTP, diameter 25 mm,
418 Millipore), respectively. Filters were rinsed twice with sterile phosphate buffered saline (PBS),
419 air-dried and stored at -20°C until further processed. The following horseradish peroxidase-
420 labeled probes were used to determine the relative proportions of specific bacterial populations:

probe mixture EUB338I-III for most *Bacteria* (Daims *et al*., 1999), BET42a for *Betaproteobacteria* (Manz *et al*., 1992), R-BT065 for the R-BT065 lineage of the *Rhodoferax* sp. BAL47 cluster of *Betaproteobacteria* (*Limnohabitans* spp.) (Šimek *et al*., 2001), PnecABCD-445 for the *Polynucleobacter* cluster of *Betaproteobacteria* (Hahn *et al*., 2005), CF319a for *Cytophaga-Flavobacteria* (Manz *et al*., 1996), HGC69a for *Actinobacteria* (Roller *et al*., 1994). CARD-FISH was performed according to Sekar and colleagues (Sekar *et al*., 2003). Subsequently, filters with hybridized bacterial cells were transferred on the slides coated with the autoradiography emulsion (NTB, Kodak, USA) and were kept at 4°C in the dark. To obtain the maximum number of cells with visible Leu incorporation while minimizing the number of background-associated grains different MAR exposure times were tested. Optimal MAR exposure times for bacteria at *in situ* and at experimental samples were 24 and 15 h, respectively. Subsequently, bacteria were stained with DAPI (final concentration 1 µg l^{-1}) and the relative proportions of hybridized cells and of cells with visible leucine incorporation were determined by epifluorescence microscopy (Axioskop, Zeiss, Germany).

Quantitative evaluation of MAR in P. rubescens

Constant MAR conditions and an exposure time of 48 h were applied to all treatments and blanks for the quantitative analysis of leucine incorporation by *P. rubescens.* Analysis was carried out using a fully automated epifluorescence microscope (AxioImager.Z1, Carl Zeiss, Germany) equipped with a motorized stage for microscopic slides, an LED epifluorescence illumination device (Colibri, Zeiss) and the filter set 62 HE (Zeiss). Imaging was performed using a CCD camera (AxioCam MRm, Zeiss) and the image analysis software AxioVision 4.6 (Carl Zeiss). Image acquisition and processing was automated within the Visual Basic for Applications module of AxioVision. Filament localization, stage positioning and setting of the camera

444 exposure time required operator actions. First, corresponding pairs of DAPI fluorescence
445 (excitation at 365 nm) and chlorophyll *a* (chl *a*) autofluorescence (excitation at 590 nm) images
446 of *P. rubescens* filaments (1 filament per image) were recorded using a 40 × oil immersion
447 objective (EC Plan-NEOFLUAR, NA = 1.3, Zeiss) for at least 10 randomly selected filaments for
448 each triplicated treatment and blank. Images were first inspected for the presence of microbes
449 associated with *P. rubescens* filaments that might affect quantitative evaluation, using the
450 combination of DAPI and chl *a* fluorescence. To quantify the leucine incorporation by individual
451 filaments of *P. rubescens*, we subsequently captured a z-stack of 20 MAR images per field of
452 view over a range of 20 µm in a transmission illumination mode. The MAR image z-stack was
453 transformed into a single, composite MAR image by a minimum intensity projection. This image
454 was then inverted and MAR grains were detected using the dynamic thresholding algorithm
455 provided by AxioVision (Zeiss). In order to distinguish between filament-associated and
456 background MAR grains on an image, the area of the filament was detected by dynamic
457 thresholding on the corresponding image of chl *a* fluorescence and dilated by 2 µm. The resulting
458 binary mask covered the theoretical area of maximal distance of electrons emitted from the
459 filament that incorporated tritium-labeled leucine. The numbers of MAR grains and the
460 cumulative MAR grain area inside and outside this binary mask were subsequently determined.
461 Subsequently, the cumulative background MAR grain area was subtracted from the
462 corresponding filament-associated MAR grain area. Results were expressed as the percentage of
463 total filament area covered by grains (MAR+ filament area).

464 *Leucine incorporation rates*

465 Assays for leucine incorporation rates were performed within 1 h after sampling.
466 Triplicate samples and paraformaldehyde-fixed blanks of 5 ml were incubated with L-[^{3}H]-

leucine (Leu, final concentration 10 nmol l^{-1}, specific activity 2.55 TBq $mmol^{-1}$, Amersham) for 2 h at *in situ* temperature and irradiance conditions and preserved in paraformaldehyde (final concentration, 2 %) afterwards. To specifically separate leucine incorporation rates of *P. rubescens* and whole microbial community, samples were filtered through either 5 or 0.2 µm cellulose filters (type GSWP, diameter 25 mm, Millipore), respectively. Filters were rinsed twice with ice-cold trichloroacetic acid and ethanol (Kirchman, 1985). Radioactivity incorporated into microbial biomass was measured in a Beckman LS50000TD liquid scintillation counter (Beckman, USA) and corrected for blanks. The amount of incorporated leucine was expressed in pmol l^{-1} h^{-1}. The same procedure was used for determining leucine incorporation rates by axenic strains of *P. rubescens* and *Limnohabitans* spp. in pure culture and co-culture incubations. In parallel, a presence of *Limnohabitans* spp. possibly attached to *P. rubescens* was inspected microscopically after staining with DAPI.

Relative fluorescence and sizing of P. rubescens

Relative phycoerythrine (excitation at 488 and emission at 576 nm) and chlorophyll *a* fluorescence (excitation at 488 and emission at 685 nm) of *P. rubescens* was determined in a volume of 0.2 ml using a Spectramax Gemini XS fluorescence microplate reader (Molecular Devices, USA). In parallel, samples of 1 to 5 ml were filtered onto the 5 µm polycarbonate filters (type GTTP, diameter 25 mm, Millipore) and the filters were inspected under an epifluorescence microscope (Olympus Provis AX 70). The abundance of *P. rubescencs* filaments in pure cultures and defined co-cultures was determined using the line-intercept method (Nedoma *et al*., 2001). The mean filament length was measured by the image analysis software LUCIA D (Laboratory Imaging, Czech Republic). Total biomass of *P. rubescens* was calculated from filament length

and abundances as described in Van den Wyngaert and colleagues (Van den Wyngaert *et al*, 2011).

Statistical analyses

Prior to analyses, all percentage data (relative proportions of major bacterial groups, and Leu+ cells) were arcsine transformed. All the transformed data fitted to a normal distribution. Differences in the relative abundances and proportions of Leu+ cells of BET, R-BT, CF, ACT and filament MAR+ area of *P. rubescens* were tested by means of a 1-way ANOVA followed by Tukey's or Dunnett's multiple comparison post-hoc tests. Specifically, we tested for significant differences (i) between the 3 depth layers in the lake (2.5, 12.5, 30 m) and (ii) in the 3 treatments (increased, ambient, dark) compared to the initial treatment. In addition, differences in abundances and leucine incorporation rates of *Limnohabitans* spp. between different irradiance levels (increased, ambient, dark) in pure cultures and in co-cultures with *P. rubescens* after 169 h of incubation as well as irradiance-related differences in abundance, mean length and biomass of *P. rubescens* were tested by a 2-way ANOVA followed by pair-wise comparisons (Bonferroni`s post-tests). All analyses were performed using GraphPad Prism (GraphPad Software).

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736 **Table 1.** Relative abundances (% of total DAPI-stained cells) and relative proportions of cells incorporating leucine (% of Leu+ cells in all
737 hybridized cells) of *Betaproteobacteria* (BET42a), R-BT065 lineage of the *Rhodoferax* sp. BAL47 cluster of *Betaproteobacteria* (R-
738 BT065), *Actinobacteria* (HGC69a), and *Cytophaga-Flavobacteria* (CF319a) in 3 selected depths (2.5, 12.5, and 30 m) of the lake water
739 vertical profile. Values are means of triplicates ± SD. Treatments with different superscripts ($^{a, b}$) were statistically distinguishable at $p < 0.05$ (tested for each bacterial group independently).

741

742		BET42a			R-BT065			HGC69a			CF319a		
743		2.5 m	12.5 m	30 m	2.5 m	12.5 m	30 m	2.5 m	12.5 m	30 m	2.5 m	12.5 m	30 m
744	Relative abundance (%)	9.2±0.8	10.1±1	10.2±0.8	2.6±0.4	2.6±0.7	2.5±0.6	21.4±1	19.8±2	17.6±2.4	9.2±1.2	9.5±0.2	8.1±1.7
745	Leu+ cells (%)	72±4.4 ^{a}	65±1.2 ^{a,b}	56±5.8 ^{b}	93±3.9 ^{a}	92±4.5 ^{a}	71±2.3 ^{b}	53±6.4	59±3.2	63±2.8	8.2±1.2	8±1.4	6.7±0.7

Table 2. Relative abundances (% of total DAPI-stained cells) and relative proportions of cells incorporating leucine (% of Leu+ cells in all hybridized cells) of *Actinobacteria* (HGC69a) and *Cytophaga-Flavobacteria* (CF319a) before (initial) and after 45h of incubation at different irradiance regimes (increased, ambient, dark), corresponding to light levels in depths of 2.5, 12.5 and 30 m, respectively. Lake water sample taken from the depth of 12.5 m represents the initial sample used in the laboratory study. Values are means of triplicates ± SD, ND – not determined. Asterisks indicate that final time points were significantly different from the respective initial time point (tested for each bacterial group independently). *$p < 0.05$

	HGC69a				CF319a			
	Initial	Increased	Ambient	Dark	Initial	Increased	Ambient	Dark
Relative abundance (%)	19.8±2	21±2.9	22.2±1	26.6±2.1*	9.5±0.2	9.6±0.5	13.6±2.8*	11.9±1.3
Leu+ cells (%)	59.2±3.2	77.7±5.8*	60.3±9.6	70.6±6.7	8±1.5	ND	ND	ND

Table 3. Filament abundance, mean filament length, and biomass of *P. rubescens* prior to (initial) and after 169 h of incubation (final) at different irradiance regimes (increased, ambient, dark), respectively. *P. rubescens* was cultivated in ALW medium in pure cultures (control) and in co-cultures with *Limnohabitans parvus* or *L. planktonicus*. Values are means of triplicates ± SD. Irradiance treatments with different superscripts ($^{a, b}$) were statistically distinguishable at $p < 0.05$ (tested for pure culture or each co-culture independently) at 169 h of incubation (Bonferroni`s pair-wise post-tests after a 2-way ANOVA).

	Initial	Final								
	P. rubescens	*P.rubescens* (Control)			*P.rubescens* + *L. parvus*			*P.rubescens* + *L. planktonicus*		
		Increased	Ambient	Dark	Increased	Ambient	Dark	Increased	Ambient	Dark
Abundance (10^3 filaments ml^{-1})	12±0.7	33.1±9^{a}	25.6±2^{ab}	14.6±3^{b}	21.7±7	18.1±4	16.9±1	26.2±11	16.2±5	14.4±4
Mean filament length (µm)	290±52	159±26^{a}	246±27^{ab}	316±54^{b}	198±46	279±53	273±32	168±16^{a}	361±87^{b}	365±37^{b}
Biomass (mg C l^{-1})	11.4±2.2	16.7±1.3^{ab}	19.8±1.4^{a}	14.4±1^{b}	13.6±2	15.9±0.8	14.5±1.1	13.9±1.7^{a}	18.3±1.4^{b}	16.4±4.3^{ab}

771 **Legends to figures**

772 **Fig. 1.** Depth profiles of temperature, oxygen concentration and photon irradiance (left panel)
773 and depth profiles of total cell numbers of bacteria, picocyanobacteria and *P. rubescens* (right
774 panel) on 17 Sept 2007. Water from a depth of 12.5 m (*P. rubescens* deep maximum) was used
775 for the laboratory study. Horizontal dashed lines at 2.5, 12.5 and 30 m correspond to increased,
776 ambient and dark irradiance conditions used in the experiment.

777

778 **Fig. 2.** Quantitative evaluation of leucine incorporation by individual filaments of *P. rubescens*
779 after microautoradiography (MAR). MAR+ filament area (as % of total filament area covered
780 with silver grains) of filaments in 3 selected depths of the lake (2.5, 12.5, and 30 m). The line
781 within the box plot marks the median. Boundaries of the box indicate the 25^{th} and 75^{th}
782 percentiles. Error bars indicate the 5^{th} and 95^{th} percentiles. Dots represent the outlying values. At
783 least 10 filaments in 10 images were analyzed per each replicate (A, B) and blank.

784

785 **Fig. 3.** Quantitative evaluation of leucine incorporation by individual filaments of *P. rubescens*
786 after MAR. MAR+ filament area (% of total filament area covered with silver grains) prior to
787 (initial) and after 45 h of incubation at 3 different irradiance regimes (increased, ambient, dark)
788 corresponding to the light levels in 2.5, 12.5 and 30 m depth, respectively. Error bars = SD.
789 Asterisks above bars indicate that increased or dark treatments were significantly different from
790 the initial treatment. $^{**}p < 0.01$

791

792 **Fig. 4.** Relative abundances (% of total DAPI-stained cells, upper panel) and relative proportions
793 of cells incorporating leucine (% of Leu+ cells in all hybridized cells, lower panel) of

Betaproteobacteria (BET42a), and the R-BT065 lineage of the *Rhodoferax* sp. BAL47 cluster of *Betaproteobacteria* (R-BT065) prior to (initial) and after 45 h of incubation at 3 different irradiance regimes (increased, ambient, dark). Values are means of triplicates, error bars = SD. Asterisks above bars indicate that increased, ambient or dark treatments were significantly different from the initial treatment. *$p < 0.05$, **$p < 0.01$

Fig. 5. Relative fluorescence of phycoerythrine and chlorophyll *a* and total leucine incorporation rates of *P. rubescens* specific to *P. rubescens* biomass (mg C l^{-1}). Initial values and data after 169 h of incubation at 3 different irradiance regimes (increased, ambient, dark) are shown. All strains were cultivated separately in ALW medium. Values are means of triplicates, error bars = SD.

Fig. 6. Total cell numbers (upper panels) and total leucine incorporation rates (lower panels) of *L. parvus* and *L. planktonicus* in pure cultures and in co-cultures with *P. rubescens*. Initial values and data after 169 h of incubation at 3 different irradiance regimes (increased, ambient, dark) are shown. All strains were cultivated in ALW medium. Values are means of triplicates, error bars = SD.

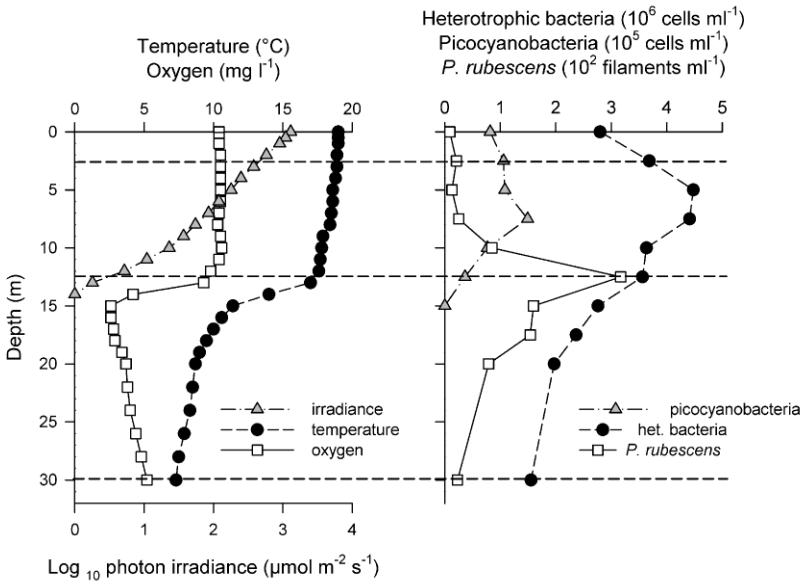

Temperature (°C)
Oxygen (mg l-1)
Heterotrophic bacteria (10^6 cells ml-1)
Picocyanobacteria (10^5 cells ml-1)
P. rubescens (10^2 filaments ml-1)
Depth (m)
irradiance
temperature
oxygen
picocyanobacteria
het. bacteria
P. rubescens
Log 10 photon irradiance (µmol m-2 s-1)

Fig.1

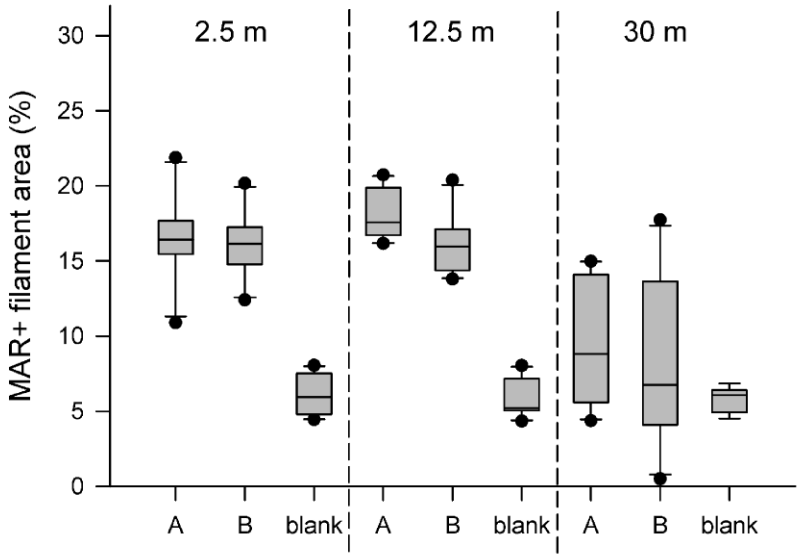
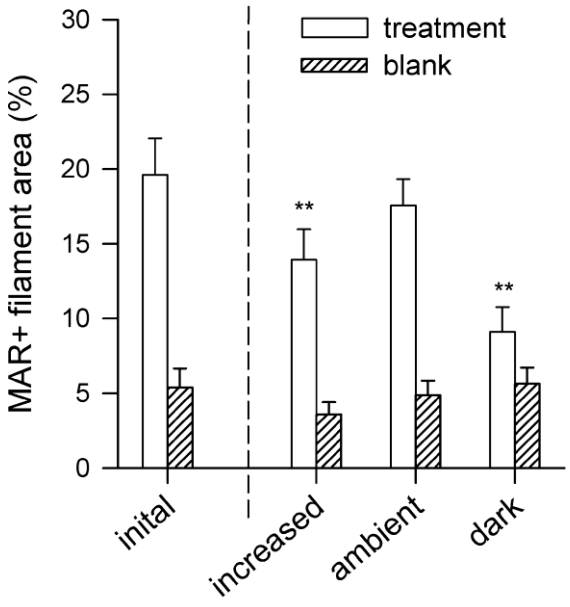

2.5 m
12.5 m
30 m
MAR+ filament area (%)
30
25
20
15
10
5
0
A
B
blank
A
B
blank
A
B
blank

Fig. 2



838

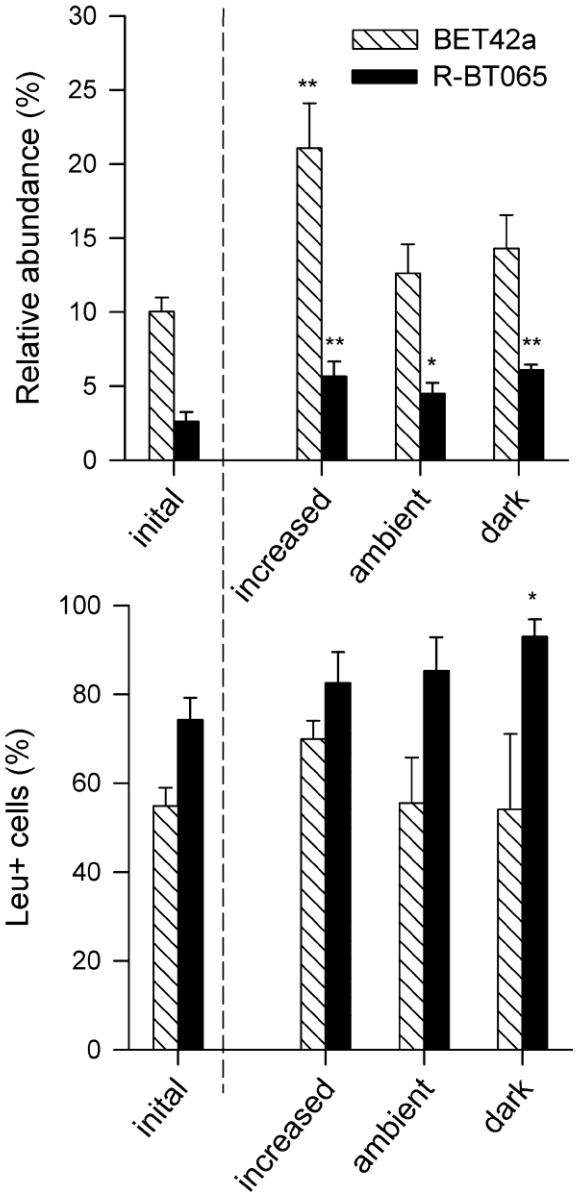
839 Fig. 3

840

841

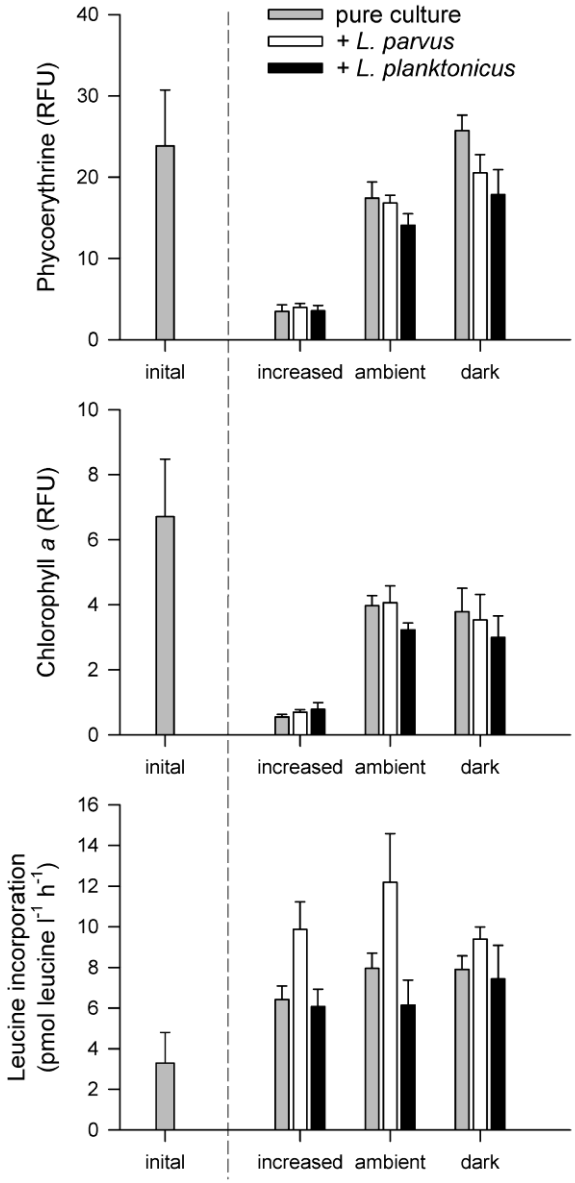
842

843


BET42a
R-BT065
Relative abundance (%)
30
25
20
15
10
5
0
**
**
*
**
inital
increased
ambient
dark
Leu+ cells (%)
100
80
60
40
20
0
*
inital
increased
ambient
dark

844

845 Fig. 4



846

847 Fig. 5

848

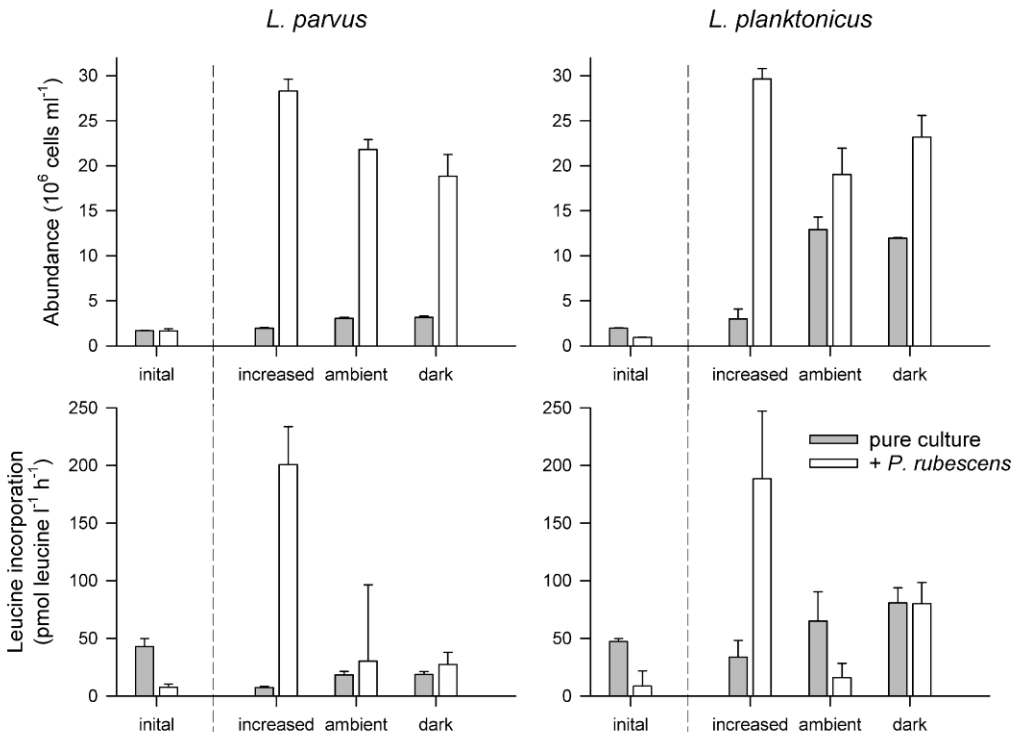

L. parvus
L. planktonicus
Abundance (10^6 cells ml^{-1})
Leucine incorporation (pmol leucine l^{-1} h^{-1})
inital
increased
ambient
dark
pure culture
+ P. rubescens

Fig. 6

Supporting information

Table S1. Results of pair-wise comparisons (Bonferroni`s post-tests after a 2-way ANOVA) of significant differences in phycoerythrine and chlorophyll *a* fluorescence and leucine incorporation rates of *P. rubescens* between different irradiance levels (increased, ambient, dark) in pure cultures and in co-cultures with either *L. parvus* or *L. planktonicus* after 169 h of incubation. Significant differences are in bold (**p < 0.05, p < 0.001**), ns – not significant

Parameter	Treatments tested	Pure cultures	Co-cultures with *L. parvus*	Co-cultures with *L. planktonicus*
phycoerythrine	increased vs. ambient	**p < 0.001**	**p < 0.001**	**p < 0.001**
	increased vs. dark	**p < 0.001**	**p < 0.001**	**p < 0.001**
	ambient vs. dark	**p < 0.001**	**p < 0.05**	**p < 0.05**
chlorophyll *a*	increased vs. ambient	**p < 0.001**	**p < 0.001**	**p < 0.001**
	increased vs. dark	**p < 0.001**	**p < 0.001**	**p < 0.001**
	ambient vs. dark	ns	ns	ns
Leu incorporation	increased vs. ambient	ns	ns	ns
	increased vs. dark	ns	**p < 0.05**	ns
	ambient vs. dark	ns	ns	ns

Table S2. Results of pair-wise comparisons (Bonferroni`s post-tests after a 2-way ANOVA) of significant differences in abundances and leucine incorporation rates of *L. parvus* and *L. planktonicus* between different irradiance levels (increased, ambient, dark) in pure cultures and in co-cultures with *P. rubescens* after 169 h of incubation. Significant differences are in bold (**p < 0.05, p < 0.01, p < 0.001**), ns – not significant

Bacteria	Parameter	Treatments tested	Pure cultures	Co-cultures
L. parvus	abundance	increased vs. ambient	ns	**p < 0.001**
		increased vs. dark	ns	**p < 0.001**
		ambient vs. dark	ns	**p < 0.05**
	Leu incorp.	increased vs. ambient	ns	**p < 0.001**
		increased vs. dark	ns	**p < 0.001**
		ambient vs. dark	ns	ns
L. planktonicus	abundance	increased vs. ambient	**p < 0.001**	**p < 0.001**
		increased vs. dark	**p < 0.001**	**p < 0.001**
		ambient vs. dark	ns	**p < 0.05**
	Leu incorp.	increased vs. ambient	ns	**p < 0.001**
		increased vs. dark	ns	**p < 0.01**
		ambient vs. dark	ns	**p < 0.05**

Fig. S1. Total leucine incorporation rates in water from Lake Zürich at depths of 2.5, 12.5 and 30 m (left panel), and before (initial) and after 45h of incubation at different irradiance regimes (increased, ambient, dark, right panel), respectively. The total fraction consists of the whole microbial community while the fraction of > 5 µm consisted mainly of *P. rubescens* filaments, other filamentous bacteria, and colonial cyanobacteria. Values are means of triplicates, error bars = SD

Fig. S2. Micrographs of *P. rubescens* (A, B), filamentous bacteria (C, D), and colonial cyanobacteria (E, F) present in the > 5 µm fraction besides *P. rubescens*. Left panels: chlorophyll *a* fluorescences (A, C), and DAPI staining (B). Right panels: leucine incorporation (B, D, F) as indicated by black grains after MAR. Bar = 20 µm

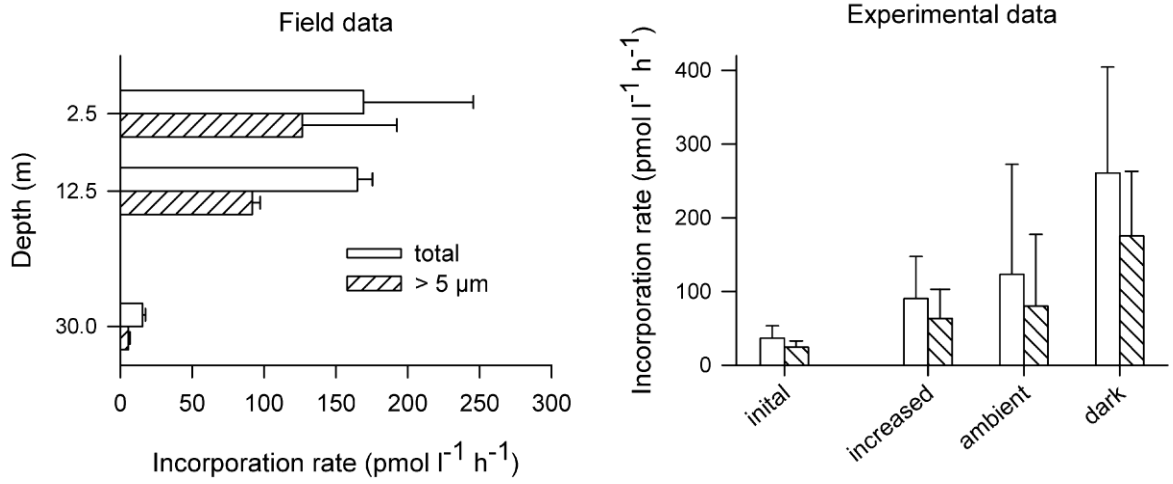

Field data
Depth (m)
2.5
12.5
30.0
total
> 5 µm
0
50
100
150
200
250
300
Incorporation rate (pmol l-1 h-1)
Experimental data
Incorporation rate (pmol l-1 h-1)
400
300
200
100
0
inital
increased
ambient
dark

Fig. S1

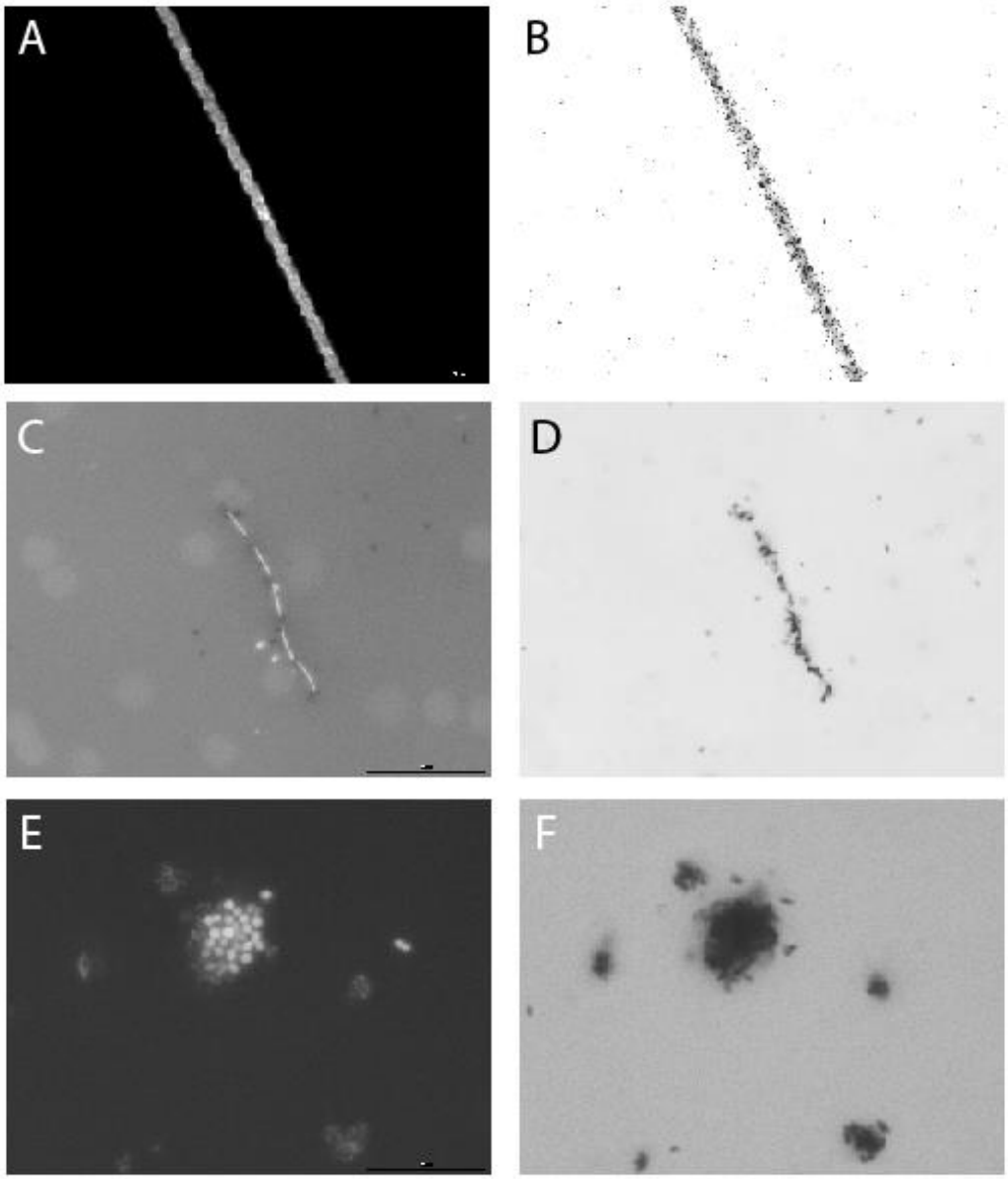


Fig. S2